Alzheimer's disease – the therapeutic era

Because of the aging of the general population, Alzheimer's disease (AD) is one of the major challenges of public health at the end of this century. However, recent therapeutic and diagnostic advances allow for a more optimistic view for this disease, which has long been considered as excluded from medical progress. The meeting that was held at the Institut Pasteur (Paris, France on 5–6 November 1998) under the subtitle *The Therapeutic Era* was thus most timely.

These advances became possible because of an improved understanding of the physiopathology of AD. Its two central markers are the senile plaques, formed essentially by the β-amyloid peptide, and the τ-proteins of neurofibrillar degeneration. Physiopathology of the disease is in fact dominated by a debate between the concepts of the 'τ-ists' and the 'β-ists' – each claiming a causal role for either the τ-proteins or β-amyloid peptide, respectively, in the disease.

The β-amyloid peptide contains 40–42 amino acids, derived by cleavage from the amyloid protein precursor (APP). Konrad Beyreuther (Center for Molecular Biology, Heidelberg, Germany) has shown that the β-amyloid peptide is the major proteinaceous component of senile plaques in AD. After observing that the antibody domain is essential for axonal delivery of APP, he suggested that it operates in the intraneuronal sorting of APP by interacting with an axonal sorting receptor. Its pathogenic function is probably exerted via the inhibition of this axonal sorting. Indeed, the major molecular defect underlying the synaptic loss and neurodegeneration in AD is a disturbance of axonal transport. An attractive hypothesis for the role of the antibody domain in intraneuronal sorting would be that APP interacts through the antibody domain directly with a sorting receptor in the cell in order to get packaged into axonal transport vesicles. The τ-protein would then play only a functional role in the physiopathology of AD. According to the β-ists, it is probable that targeting APP expression, transport, and metabolism leading to antibody generation and aggregation will lead to effective therapeutic strategies. Beyreuther also showed during the meeting that a 70% reduction of the intracellular cholesterol by lovastatin and by methyl-β-cyclodextrin suppresses the formation of β-amyloid peptide, which might explain the beneficial effect of oestrogens in AD.

Neurofibrillar degeneration involves the hyperphosphorylation of ~80% of τ-proteins. Phosphorylation occurs preferentially on serine or threonine residues and is favoured by glycogen synthetase kinase-3 (GSK3). According to Hugo Geerts (Janssen Research Foundation, Beerse, Belgium), GSK3 would play a central role by inducing phosphorylation of τ-proteins and of β-catenin. Video-microscopy shows that a moderate hyperphosphorylation reduces the rapid axonal transport in a culture of hippocampic neurones and that the retraction of the neurites leads to the implosion of cell bodies of the hyperphosphorylated neuroblastomes. The discovery of families with muted genes for the τ-protein on chromosome 17 and of a fronto-temporal dementia shows that the presence of a τ-protein alone may explain the cognitive disorder. How the presence of the β-amyloid peptide may induce the phosphorylation of the τ-protein has not been clearly determined. The description of the different brain areas affected sequentially by the neurofibrillar degeneration allows classification of this progression in ten stages – AD being recognized at stage 7, characterized by the involvement of prefrontal, inferior parietal and superior temporal cortex areas.

Risk factors

Jean-François Dartigues (INSERM U330, Bordeaux, France) discussed the risk factors for AD as detected by the PAQUID (Personne Agée QUID), a French population-based prospective cohort study conducted in the Bordeaux area and designed to study the normal and pathological brain aging of people aged 65 and over. The annual incidence of dementia was higher in women than in men (1.3 versus 1.17%). The PAQUID and the Rotterdam study by Albert Hofman (Erasmus University Medical School, Rotterdam, The Netherlands) disagree on the effect of cultural and social factors. According to PAQUID, the risk factors for dementia correlate with living in a village where the aluminium concentration in drinking water is >100 mg ml^{-1}, whereas isolation and a moderate wine intake would have a protective effect. The results of the Rotterdam study demonstrate a role of vascular risk factors (diabetes, auricular fibrillation, smoking habits, arterial rigidity and carotid pathologies) for dementia and AD.

In her presentation, Christine Van Broeckhoven (University of Antwerp, Belgium) highlighted the importance for the early onset dementia of mutations at three genes [that is, for APP (chromosome 21), and the preselenins 1

1359-6446/99/$ – see front matter PII: S1359-6446(99)01318-5

(chromosome 14) and 2 (chromosome 1)] that lead to amyloid accumulation. The only recognized risk factor for late onset AD is the E4 allele of apolipoprotein E (ApoE4) of chromosome 19. The relative risk associated with the presence of ApoE4 is estimated to be fourfold higher than with the E3 genotype, but an interaction with the score of vascular lesions is also reported.

Role of acetylcholine

Present therapeutic progress in AD is mostly based on hypotheses concerning the role of the cholinergic deficit in memory deficiency. According to Ezio Giacobini (University of Geneva, Switzerland), the cholinergic system affects the production of APP and neuroprotection. Clinical assays throughout the world have pointed to clinical improvement following the administration of cholinesterase inhibitors (ChEI), which augment the concentrations of acetylcholine in the brain. The clinical effect of most ChEIs in AD seems to be stabilization of the symptomatology, rather than improvement of disease conditions from the baseline. Assays began in the 1970s with physostigmine, followed by tacrine in the 1980s; other drugs are in development or have just been released. As pointed by Elkan Gamzu (Cambridge NeuroScience, Cambridge, MA, USA), an evolution is noted between the Summers assay on tacrine, of 1986, with just over 20 patients and the large and methodologically sound present trials involving hundreds of patients. Despite this, the development of tacrine was hindered by the absence of guidelines, by an inappropriate methodology based on crossovers, and on a phase of 'enrichment' for responder selection. This phase of development of tacrine was encouraged by the FDA and had positive effects on the elaboration of an internationally recognized methodology based entirely on psychometric and clinical evaluation and on the standardization of inclusion criteria. After analysing 30 Phase III trials involving 6000 patients, Giacobini noted that three irreversible or pseudo-irreversible ChEIs (heptagastine, rivagastine and metrifonate) used for six months, displayed a similar potency, but rather clinical stabilization than clear-cut improvement. Differences are noted between these drugs with respect to pharmacodynamics, selectivity and receptor affinity. It is likely that the frequency and the intensity of their undesirable effects will determine their market position. Donezepil (Aricept) was the first marketed selective ChEI, its efficacy having been shown at 5–10 mg per day, as indicated by the ADAS-cog and on the clinician's interview-based impression of change with care-giver information (CGIC, Clinical Global Impression of Change).

After undergoing ~40 clinical trials and being evaluated by different scales, rivastagine (Exelon) was recently launched at 6–12 mg. The prodrug Metrifonate (Bayer) has a long duration of action; its efficacy was tested over 30–80 mg per day, and it improved significantly most of the psychiatric disturbances detected by the Neuropsychiatric Inventory. Co-lateral effects delayed its development. Reminyl (Galantamine, Janssen) is presently under investigation, and more than 2000 patients have been included in Phase III trials that show efficacy at 24 and 32 mg per day for six months.

It has also been suggested to treat AD with muscarinic agonists, or agonists specific for M1 cholinergic receptors, which might be devoid of colateral effects. Such a compound, SB202026 (Memrin, SmithKline Beecham), appears to be somewhat efficient against delirium and hallucinations.

Other therapeutic approaches

The utilization of nonsteroidal anti-inflammatory drugs (NSAID) is based upon the recognition that progression of AD is accompanied by inflammation, involving astrocytes and microglia associated to senile plaques and to neurofibrillar degeneration. Retrospective studies have suggested a protective effect of NSAID. Difficult problems are nevertheless raised by the utilization of COX-2 inhibitors in AD, as discussed by Jean-Marc Orgogozo (Université de Bordeaux, France).

Propentofyline (Hoechst) has an original mode of action; it interferes with neuro-inflammation related to the activation of glial cells in AD and vascular dementia – that is, with degeneration. Present trials involve phases of early and differed withdrawal in order to start testing.

As discussed by Laura Bossi (Sanofi Recherche, Gentilly, France), the neurotrophic approach of AD may be promising, because neurotrophic factors stimulate differentiation and survival of certain neurons. Nerve growth factor (NGF) is the main lead of neurotrophines, which include brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and NT4/5. NGF and NT3 were assayed in AD. The compound SR577746A has shown an effect of neurotrophin secretion and improvement of memory disturbances, and might be a candidate for preventing AD. In addition, ensaculine is a novel benzopyrone that activates *N*-methyl-D-aspartate receptors displaying neurotrophic effects. It shows some efficacy in animal models of memory and shows tolerance in human males.

Françoise Forette (Hôpital Broca, Paris, France) discussed primary prevention of AD, particularly with respect to oestrogen and antihypertensive therapy. A few retrospective studies have suggested that treating menopause with oestrogens might reduce the risk for AD, but there does not appear to be a consensus on the matter. The hypothesis was supported, however, by results of prospective studies showing a 40–50% reduction of AD in the treated

group. Nevertheless, the selection bias used in this sort of study requires the use of randomized trials to demonstrate the efficacy of substitutive oestrogens in the primary prevention of AD.

An antihypertensive treatment prevents dementia according to evidence in the Systeur Dementia Project. The incidence of AD-type dementia rate was significantly lower in a group treated with nitrendipine, as first line antihypertensive drug, than in the placebo group, suggesting a neuroprotective effect by some types of calcium inhibitors.

If one considers the hypothesis of the primary pathogenic role of the β-amyloid peptide in AD, it might be useful to destroy this protein therapeutically, as stated by Einar M. Sigurdsson (New York University Medical Center, NY, USA). A 'β-sheet breaker' that dissociates already formed peptides was shown to prevent the destruction of rat neurons by antibody and to reduce microglia inflammation around the deposits of antibody. This may be a promising treatment to prevent or reduce the amyloid brain lesions of AD.

Overall, this Institut Pasteur EurΩconference presented several forms of treatment for AD and allowed a vivid debate concerning methodologies to improve AD trials.

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Pharmacogenetic-oriented drug development

Drug pharmacokinetics and target interactions vary among individuals, sometimes leading to limited efficacy or adverse reactions [Lichter, J.B. *et al.* (1997) *Curr. Opin. Biotechnol.* 8, 692–695]. These variable reactions are sometimes more common among members of certain ethnic groups than in others [Bertilsson, L. *et al.* (1997) *Acta Psychiatr. Scand.* (Suppl.) 391, 14–21]. A large segment of drug development today is carried out in the USA, Canada and Europe. Consequently, many drug trials recruit largely Caucasian subjects. This is particularly true during Phase I trials, which often enlist few healthy subjects at a single clinical center. The resulting under-representation of minority groups in the early-phase trials sometimes leads to efficacy and adverse reaction problems later, often arising in subjects from such minority groups.

Polymorphic sites and drug design

Variable levels of drug efficacy and side effects often reflect a polymorphic site in a gene coding for one of the drug's metabolizing enzymes, such as a sulphatase [Tomatsu, S. *et al.* (1998) *Hum. Mutat.* (Suppl.) 1, S42–S46] or a methyltransferase [Preuss, C.V. *et al.* (1998) *Mol. Pharmacol.* 53, 708–717]. Polymorphic sites in drug metabolizing enzymes could have powerful pharmacokinetic impact. The polymorphic sites need not be situated in a coding region of the gene; intronic polymorphic sites could result in large expression alterations, leading to robust effects on drug clearance. For example, the expression of tyrosine hydroxylase is strongly affected by an intronic tetranucleotide polymorphic microsatellite sequence [Meloni, R. *et al.* (1998) *Hum. Mol. Genet.* 7, 423–428]. In other instances, the variability may reflect a polymorphic site in the gene coding for the drug's target protein itself, such as a membrane receptor or ion channel, so that certain individuals would be less responsive to the drug, or in other instances, too sensitive to its consumption.

Such adverse effects, if not detected early during drug development, could become serious obstacles during later phases. The best way to combat the reduced efficacy and adverse reactions arising in some individuals, caused by the inherently large human genetic variation, is to recruit subjects from several ethnic groups for the clinical trials. However, this is often an unrealistic solution, given the already complex setup of drug trials. When the genes coding for the drug target and its metabolizing enzymes are known, a simpler and more modest solution is feasible: studying the polymorphism of the relevant genes in a large collection of DNA samples of individuals from a diverse ethnic background. In this way it may become possible to detect in advance polymorphic sites in the relevant genes, which may affect the drug's interaction with its target protein or with its metabolizing enzymes [Linder, M.W. *et al.* (1997) *Clin. Chem.* 43, 254–266].

Proteomics and pharmacogenetics

Proteomics – the study of protein expression under diverse situations and in

1359-6446/99/$ – see front matter PII: S1359-6446(99)01329-X